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Inoculation of Living Aspen Trees with Basidiospores of Fomes igniarius var. populinus

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ABSTRACT

Basidiospores of *Fomes igniarius* var. *populinus* placed in sapwood wounds of aspen (*Populus tremuloides*) 5-13 cm in diam germinated well (30-35%) in fresh wounds and two-day old wounds (24-32%), very slightly (1-3%) in four-day old wounds, and not at all in six-day or older wounds. Basidiospores placed in wounds during April and May did not germinate, but those placed in July did.

Published accounts of infection of aspen (*Populus tremuloides* Michx.) by *Fomes igniarius* (L. ex Fr.) Kickx var. *populinus* (Neuman) Campbell usually state that dead, broken stubs provide a direct passage for the decay-causing fungus through the supposedly resistant sapwood into the heartwood (1, 6, 7), or that any wound in which heartwood is exposed will allow the entrance of the fungus (5). Although Etheridge (2) attempted to show that dead branches were the invasion site, *F. igniarius* was never cultured from dead branches except as a lateral extension of a heartwood infection. Schmitz and Jackson (4) stated that theoretically every branch scar on an aspen tree is a possible infection court for invasion by *F. igniarius*, and that infection is associated also with fire scars and insect injuries. Good and Spanis (3), from results they obtained in the laboratory, suggested that old rather than fresh wounds are the site of infection. They also suggested that infection probably takes place in the spring or fall and not during the summer, because of lower spore production in the summer and less suitable relative humidity.

Since there are no reports in the literature of inoculations of aspen trees in the field with basidiospores of *F. igniarius*, there has been only speculation as to when and where the infection takes place in nature. The following describes the results of inoculating aspen with basidiospores.

F. igniarius basidiospores were collected on clean glass slides placed in plastic boxes attached to the tree around sporulating conks in the field. Spores were collected during a 12- to 16-hr period starting at 5-8 PM. Only those spore collections that resulted in a heavy white cast on the slides were used for inoculation. The freshly collected spores from a single conk were washed from the slides with sterile, distilled water just before inoculating the trees. One fourth to 1 ml of spore suspension (1-8 million spores/ml) was placed in each wound made with a Swedish increment hammer or a 5-mm drill.

Inoculations were made in fresh wounds and in wounds 1 week old in aspen trees 5-10 cm in diam and 12 years old, on 1, 8, and 15 July 1966, 3, 4, and 10 August 1966, 22 and 29 April 1967, and 13 and 20 May 1967 at the University of Minnesota Cloquet Forest Research Center. Another set of inoculations was made in 0-, 2-, 4-, 6-, and 8-day-old wounds in aspen 13 cm in diam and 15 years old on 3 July 1967, near Alexandria, Minn.

One week after inoculation, some of the trees were cut and the sections including the wounds were placed in plastic bags and frozen at −20 C. Additional trees were cut 2, 3, 4, and 12 weeks after inoculation.

Tangential sections 16 μ thick, at 2, 10, and 20 mm from the cambium, were cut using a cryostat at −20 C. These sections included the lower half of the circular wound and 10 mm of the wood below the wound (Fig. 1). The trees from Alexandria, in contrast to those from Cloquet, were sectioned at distances of 2, 25, and 50 mm from the cambium. The sections were stained with lactophenol cotton blue and the slides examined at a magnification of ×300-500 (Fig. 2). The percentage of spores which had germ tubes, based on at least 50 spores for each area sectioned, was determined.

To ascertain whether the fungus was present and living in the wounds or in the vicinity of the wound

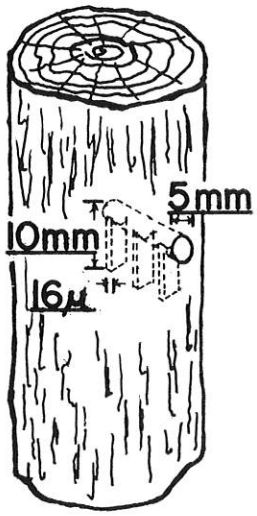

Fig. 1. Diagram of the wound and the position of the 16-μ sections that were examined microscopically.

TABLE 1. Average percentage germination of *Fomes igniarius* var. *populinus* basidiospores in fresh wounds in sapwood of aspen in the field, after 7 days, 2, 10, and 20 mm from the cambium

	Date of inoculation										
	April[a]		May[a]			July[b]			August[c]		
Distance from cambium	22	29	6	13	20	1	8	15	3	4	10
mm	%	%	%	%	%	%	%	%	%	%	%
2	0	0	0	0	0	26 bc[d]	23 c	19 c	29 bc	0	0
10	0	0	0	0	0	22 c	29 bc	61 a	41 b	0	0
20						12	31 bc	63 a	31 bc	0	0
Temperature (F)											
Average daily maximum	48.2	45.1	57.0	65.5	62.8	82.7	86.2	81.1	76.7	75.7	71.2
Average daily minimum	24.0	26.0	31.2	34.4	36.0	59.7	58.7	51.2	54.8	55.2	50.0
Precipitation											
Total inches	0.0	0.6	0.7	0.0	0.3	1.9	2.7	0.9	2.5	2.5	1.0

[a] Averages of the results from four inoculations, two in aspen saplings and two in aspen suckers.
[b] Averages of the results from six inoculations.
[c] Averages of the results from 12 inoculations.
[d] Values followed by a common letter are not significantly different using a 5% Duncan's multiple range test.

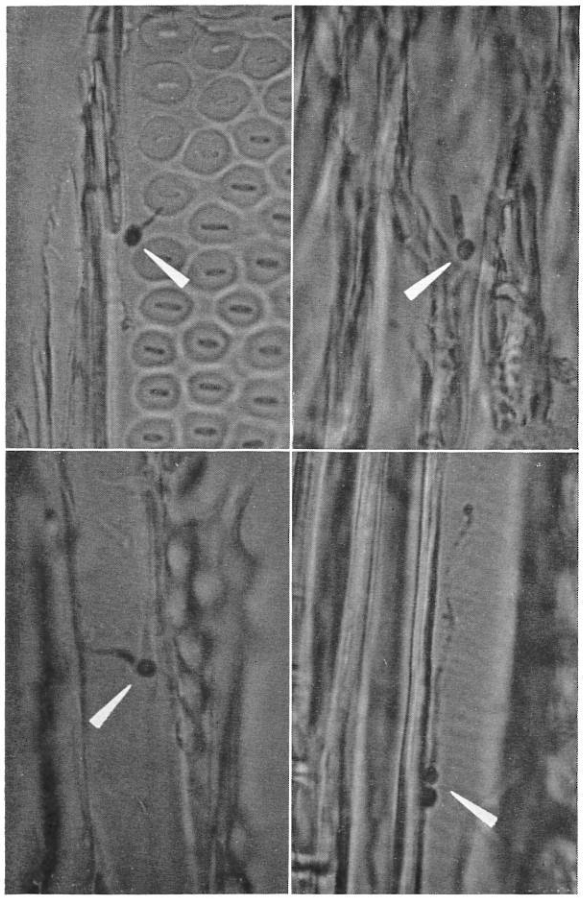

Fig. 2. Germinated basidiospores of *F. igniarius* var. *populinus* in fresh wounds in sapwood region of aspen, 1 week after the living tree had been inoculated with spores.

after 1 week to 3 months, pieces of wood tissue were cultured on 2% malt agar.

The percentage germination of spores placed in fresh wounds in the summer of 1966 and in the spring of 1967 (Table 1) includes only data from trees cut 1 week after inoculation, since after 2 weeks there was so much contamination and miscellaneous mycelium that it was difficult to find germinated basidiospores of *F. igniarius*. None of the spores germinated in wounds 1 week old. The basidiospores of *F. igniarius* germinated best in fresh wounds or those only 2 days old (Table 2).

The microorganisms isolated from tissues in the vicinity of the wounds during the period of time from 1 to 3 weeks after inoculation included *F. igniarius*, *Ceratocystis fimbriata* Ell. & Halst., *Ceratocystis serpens* (Goid.) C.Moreau, *Rhizopus* sp., *Papularia* sp., *Alternaria* sp., *Candida* sp., *Penicillium* sp., *Cladosporium* sp., bacteria, and yeasts. The microorganisms cultured from the sapwood at least 1.3 cm below the wounds 1-3 months after inoculation included the above

TABLE 2. Average percentage germination of *Fomes igniarius* var. *populinus* basidiospores, after 7 days, 2, 25, and 50 mm from the cambium in 0-, 2-, 4-, 6-, and 8-day-old wounds of aspen in the field, based on five inoculations for each age of wound

Distance from cambium	Age of wound (days)				
	0	2	4	6	8
mm	%	%	%	%	%
2	31 a[a]	32 a	1 b	0	0
25	35 a	28 a	3 b	0	0
50	30 a	24 a	3 b	0	0

[a] Values followed by a common letter are significantly different using a 5% Duncan's multiple range test.

plus three unknown Basidiomycetes, *Verticillium* sp., *Cytospora* sp., *Phoma* sp., *Streptomyces* sp., and *Coniothyrium* sp. Because of the number of contaminants and the speed with which they grew, culturing is not a reliable method of determining whether *F. igniarius* has become established. One culture of *F. igniarius* was recovered from a sapwood chip taken about 2.5 cm below a wound made and inoculated on 3 August. This tree was harvested on 10 October. *F. igniarius* was commonly isolated 1 week after the wounds were inoculated in the spring of 1967 even though no germinated basidiospores were found by microscopic examination. Apparently, these basidiospores that did not germinate remained viable for at least 1 week when placed in sapwood wounds.

These observations indicate that *F. igniarius* basidiospores germinate in relatively fresh sapwood wounds during the summer. More time is required to prove that the basidiospores of *F. igniarius* placed in aspen sapwood will cause infections leading to heart rot.

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